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Number 23

NTP Technical Report on Toxicity Studies of

o-, *m*-, and *p*-Nitrotoluenes

(CAS Nos.: 88-72-2, 99-08-1, 99-99-0)

Administered in Dosed Feed to F344/N Rats and B6C3F$_1$ Mice

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The NTP Report on the toxicity studies of *o*-, *m*-, and *p*-nitrotoluenes is based primarily on 2-week and 13-week studies that began February 9, 1988, and ended August 17, 1989, at Hazleton Laboratories of America, Rockville, MD.

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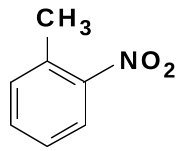
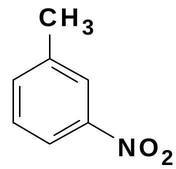


***o*-Nitrotoluene**
CAS No. 88-72-2



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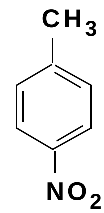
CAS No. 99-08-1



***p*-Nitrotoluene**
CAS No. 99-99-0

Molecular Formula: $C_7H_7N0_2$
Molecular Weight: 137.13

Synonyms: *o*-NT, 2NT, 2-nitrotoluene, 2-methylnitrobenzene, 2-nitrotoluol;
m-NT, 3NT, 3-nitrotoluene, 3-methylnitrobenzene, 3-nitrotoluol
p-NT, 4NT, 4-nitrotoluene, 4-methylnitrobenzene, 4-nitrotoluol

Nitrotoluenes are high production volume chemicals used in the synthesis of agricultural and rubber chemicals and in various dyes. Because of differences in the metabolism of the 3 isomers and their capability to bind to DNA, comparative toxicity studies of *o*-, *m*-, or *p*-nitrotoluene were conducted in F344 rats and B6C3F$_1$ mice. Animals were evaluated for histopathology, clinical pathology, and toxicity to the reproductive system. The nitrotoluenes were also studied in several *in vitro* and *in vivo* assays for genetic toxicity.

In 14-day studies, *o*-nitrotoluene, *m*-nitrotoluene, or *p*-nitrotoluene was administered in the feed to male and female rats and mice at concentrations ranging from 388 to 20000 ppm (5 animals/chemical/species/sex/dose). There were no effects on survival or clinical signs of toxicity in these studies, although animals at the higher doses showed decreases in body weight gains relative to controls.

In the 13-week studies, *o*-, *m*-, or *p*-nitrotoluene was given to male and female rats and mice (10 animals/chemical/species/sex/dose) in the feed at concentrations between 625 and 10000 ppm. The estimated daily doses based on measures of feed consumption were 40 to 900 mg nitrotoluene/kg body weight/day for rats and 100 to 2000 mg/kg/day for mice and were similar for each of the 3 isomers when compared for each dietary level/sex/species. There were no effects on survival in any of the studies, and clinical signs of toxicity were limited to decreases in feed consumption. Decreased body weight gains occurred in dosed rats and mice in all studies at the higher dose levels and were most pronounced in rats receiving *o*-nitrotoluene.

In rats, histopathologic analyses after 13 weeks of dosing showed toxicity to kidney, spleen, and testis in animals receiving any of the 3 isomers, and toxicity to the liver and mesothelium in male rats given *o*-nitrotoluene. Kidney toxicity observed in male rats was characterized by the presence of hyaline droplets in tubular epithelial cells, attributed to an increase in the level of α-2u globulin. Pigment, possibly lipofuscin, and karyomegaly in the *p*-nitrotoluene study were

present in the renal tubular epithelium of dosed male and female rats. In the spleen of treated male and female rats, there was a mild increase in hematopoiesis, hemosiderin deposition, and/or congestion; this effect was most severe with the para-isomer, followed by the ortho- and then the meta-isomer. Administration of *o*-, *m*-, or *p*-nitrotoluene impaired testicular function of the rat, shown by degeneration of the testis and reduction in sperm concentration, motility, and spermatid number. All 3 isomers increased the length of the estrous cycle in rats. Hepatic toxicity was characterized by cytoplasmic vacuolization and oval cell hyperplasia and by an increase in the level of serum bile acids, SDH, and ALT activities in male rats given *o*-nitrotoluene. There was no histopathologic evidence for liver toxicity in male or female rats with the *m*- or *p*-isomers, or in female rats with the *o*-isomer; but evidence of liver injury was observed in these groups, indicated by increases in relative liver weights and elevations in bile acids and liver enzymes in serum. Mesotheliomas of the tunica vaginalis were observed in 3/10 male rats receiving *o*-nitrotoluene at 5000 ppm, and mesothelial cell hyperplasia was observed in 2/10 male rats receiving *o*-nitrotoluene at 10000 ppm.

The only histopathologic evidence for toxicity in mice in the 13-week studies occurred in the olfactory epithelium in mice receiving *o*-nitrotoluene, where the chemical caused degeneration and metaplasia. No liver lesions were noted in mice, but the 3 isomers caused increases in relative liver weights. There was no toxicity to the reproductive system in male or female mice treated with any of the nitrotoluene isomers.

The 3 nitrotoluene isomers were not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98. Only *p*-nitrotoluene induced chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells, and this required metabolic activation. Sister-chromatid exchanges were increased in CHO cells following exposure to each isomer; the requirement for metabolic activation varied. Only *p*-nitrotoluene was studied in the mouse lymphoma L5178Y test; it caused mutations with metabolic activation. Unscheduled DNA synthesis (UDS) was increased in *in vitro* incubations of hepatocytes isolated from both sexes of rats and mice after receiving a single *in vivo* oral dose of *o*-nitrotoluene. UDS was not increased in a similar study with male rats given *m*- or *p*-nitrotoluene. *o*-Nitrotoluene also induced s-phase DNA synthesis in hepatocytes of rats but not in those of mice.

In summary, the 3 nitrotoluene isomers were toxic to the kidney, spleen and/or reproductive system in rats; *o*-nitrotoluene also caused lesions in the liver of male rats. No treatment-related lesions were noted in mice except with *o*-nitrotoluene where olfactory epithelium degeneration occurred. The increase in relative liver weights and the increase in UDS in liver indicate that all 3 isomers affected the liver of female rats and of male and female mice, even though histopathologic lesions were not observed. In general, the extent of the toxicity was most severe with the *o*-isomer in both rats and mice. *o*-Nitrotoluene was carcinogenic in male rats in 13-week studies, based on the occurrence of mesothelioma and mesothelial cell hyperplasia in dosed groups.

Summary of Selected Treatment-Related Effects in the 13-Week Nitrotoluene Studies

	o-Nitrotoluene		*m*-Nitrotoluene		*p*-Nitrotoluene	
	Male	Female	Male	Female	Male	Female
RATS						
Final Body Weight (90% or less than control)	↓(3) [a]	↓(3)	↓(5)	↓(5)	↓(5)	↓(5)
Liver						
Relative weight	↑(1)	↑(1)	↑(5)	↑(5)	↑(4)	↑(5)
ALT	↑(4)	–	–	↑(4)	–	↑(5)
SDH	↑(3)	–	–	–	–	–
Bile Acids	↑(4)	↑(5)	↑(4)	↑(5)	↑(5)	–
Nonneoplastic lesions	+(3)	–	–	–	–	–
Kidney						
Relative weight	↑(3)	↑(2)	↑(5)	↑(4)	↑(4)	↑(5)
Nonneoplastic lesions	+(2)	+(3)	+(1)	–	+(1)	+(1)
Spleen						
Hematology	(3)	(3)	(4)	(4)	(3)	(3)
Nonneoplastic lesions	+(2)	+(3)	+(3)	+(3)	+(1)	+(1)
Testis						
Spermatid count	↓(4)		↓(5)		↓(5)	
Nonneoplastic lesions	+(4)		+(5)		+(5)	
Mesothelium						
Neoplastic and preneoplastic lesions	+(4)		–		–	
Estrous cycle length		↑(5)		↑(4)		↑(5)
MICE						
Final Body Weight (90% or less than control)	↓(3)	↓(3)	↓(5)	↓(5)	↓(5)	↓(5)
Nose						
Nonneoplastic lesions	+(2)	+(2)	–	–	–	–
Liver						
Relative Weight	↑(3)	↑(2)	↑(1)	↑(1)	↑(1)	↑(1)

a Lowest dose group in which an effect was seen; 1 = 625 ppm; 2 = 1250; 3 = 2500; 4 = 5000; 5 = 10000.
\+ Presence of treatment-related histopathology.

PEER REVIEW

Peer Review Panel

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of *o*-, *m*-, and *p*-nitrotoluenes on November 21, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report fully and clearly presents the experimental results and conclusions.

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Summary of Peer Review Comments

Dr. J.K. Dunnick, NIEHS, introduced the short-term toxicity studies of *o*-, *m*-, and *p*-nitrotoluenes by reviewing the uses and rationale for study, the experimental design, and the results.

Dr. Goodman, a principal reviewer, said the report was written well and the results clearly presented. He stated that the rationale behind the use of each of the genetic toxicology tests employed should be presented and there should be some discussion regarding results. He suggested that a specific subsection of the Discussion could be devoted to genetic toxicology. Dr. Dunnick reported that in collaboration with Dr. E. Zeiger, NIEHS, the genetic toxicology section would be upgraded and expanded.

Dr. Davidson, a second principal reviewer, said the report did a good job of presenting background information and summarizing the results. She commented that although the degree of toxicity of the *ortho*-isomer is compared with the other 2 isomers, the *meta* and *para* isomers are not compared with each other regarding relative toxicity. Dr. Dunnick agreed that such a comparison should be added to the Abstract. Dr. Davidson noted that considering that the main uses of nitrotoluenes are in the agricultural, rubber and dye industries, it would relevant to state how occupational groups (machine operators, welders, cutters, etc.) are exposed to the chemicals. Dr. Janet Haartz, NIOSH, said the only isomer for which occupational data is available is the *para*-isomer. There were no listings for the *meta*- and *orth*-isomers.

Seeing no objections, Dr. Klaassen accepted the report, with the suggested editorial and other changes, on behalf of the panel.